Computational and Structural Biotechnology Journal 20 (2022) 6011–6022

COMPUTATIONAL ANDSTRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

Demystifying extrachromosomal DNA circles: Categories, biogenesis, and cancer therapeutics

Manrong Wu [a,b], Kunal Rai [a,b,*]

[a] Graduate Program in Quantitative and Computational Biosciences, Baylor College of Medicine, Houston, TX, USA
[b] Department of Genomic Medicine, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

ARTICLE INFO

Article history:
Received 10 July 2022
Received in revised form 21 October 2022
Accepted 21 October 2022
Available online 26 October 2022

Keywords:
eccDNAs
eccDNA classification
Biogenesis
Physiological response
Cancer therapeutics

ABSTRACT

Since the advent of sequencing technologies in the 1990s, researchers have focused on the association between aberrations in chromosomal DNA and disease. However, not all forms of the DNA are linear and chromosomal. Extrachromosomal circular DNAs (eccDNAs) are double-stranded, closed-circled DNA constructs free from the chromosome that reside in the nuclei. Although widely overlooked, the eccDNAs have recently gained attention for their potential roles in physiological response, intratumoral heterogeneity and cancer therapeutics. In this review, we summarize the history, classifications, biogenesis, and highlight recent progresses on the emerging topic of eccDNAs and comment on their potential application as biomarkers in clinical settings.

Contents

* Corresponding author.

https://doi.org/10.1016/j.csbj.2022.10.033

1. Introduction

The ring/disk shaped atypical chromosomes in *Crepis tectorum* [1] and maize[2] have long been reported in the early 1930's. Unlike ordinary rod-like chromosomes, these chromosomal derived extrachromosomal circular DNAs (eccDNAs) were modified in their organization and number in different cells, yielding in a typical variegation. Several decades later, Yasuo Hotta and Alix Bassel discovered various sizes of eccDNAs in isolated wheat nuclei and boar sperm by sedimentation analysis and electron microscopy[3], which provided one of the early evidences to support Stahl's idea that DNA might be circularized in higher organisms [4]. Contemporaneously, Cox *et al.* encountered various number of small double chromatin bodies neighboring intact chromosomes while karyotyping embryonic tumors and bronchial carcinoma tumor[5], which enhanced the credibility of the existence of small double fragments reported precedingly in a primary lesion of medulloblastoma[6]. Although it was unrealizable to trace their origin to any chromosomes, Cox *et al.* suggested the acentric ring-like chromatin bodies were not caused by random chromosomal fragmentation[5]. The foremost discovery of eccDNAs was later recapitulated in several other organisms such as the fly[7], hamster[8], mice[9], yeast[10], roundworms[11], pigeons[12], and Arabidopsis[13], suggesting that eccDNAs are prevalent and likely influence cellular processes in eukaryotic cells.

While eccDNAs have been identified in both normal and cancer cells, variations in their size distribution [14,15] and frequencies have been reported[16,17]. In general, circular structures as large as 10^4-10^7 base pairs that carry oncogenes were rarely detected in normal tissues, whereas smaller structures such as small polydispersed circular DNAs (spcDNA) were found in both normal and cancer cells[16], albeit their amount was lower in healthy individuals. Previous attempts to identify and resolve the complex eccDNA elements were constrained by low throughput methods [18]. For example, while both electron microscopy and metaphase 4′,6-diamidino-2-phenylindole (DAPI) could recognize the intactness of these molecules, they were compensated for their low sensitivity and their inability to resolve molecular architecture. Recent advances in next-generation sequencing technologies and third-generation sequencing platforms have revolutionized the way researchers decipher the complex genetic landscape of eccDNAs. Using whole genome sequencing (WGS), cytogenetic and semi-automated image analyses, Turner *et al.* identified eccDNAs in approximately half of the 17 different cancer types tested, howbeit their frequencies varied based on the tumor types[19]. Similarly, more recently, Kumar *et al.* used chromatin accessibility assays (i.e. ATAC-seq) to discover thousands of eccDNAs in various cancer types, which were further validated by inverse PCR and metaphase fluorescence in situ hybridization (FISH)[20]. EccDNAs can also be identified by Circle-seq purification and enrichment[21] coupled with long-read sequencing technologies such as long-read Nanopore and single-molecule real-time sequencing (SMRT-seq)[22]. Further, another method called CRISPR-CATCH, which does not require DNA amplification to purify targeted megabase-sized eccDNAs, was invented to overcome limitations of Circle-seq (such as the need for intact DNA circles and the fragility of large eccDNAs)[23]. More recently, a third-generation sequencing technology-based method was developed to enable detection of eccDNAs at a single-cell whole-genome level[24]. Together, the use of parallel paired-end next-generation sequencing by these studies suggest that the architecture of eccDNAs are significantly more complex than previously considered.

1.1. Classification and biogenesis of extrachromosomal DNA species

EccDNAs are categorized into multiple groups depending on their size and sequence[25], the four[26] that are commonly found in cancer are described below and their method of detection has been summarized in Table 1.

1.1.1. Small polydispersed circular DNA (spcDNA)

Despite their discovery and isolation based on their buoyancy in alkaline solutions[27], these heterogeneously sized DNA species ranging from 0.2 to 2 μm remained unnamed until 1972, when their name was coined by Smith *et al.*[28]. Since then, various spcDNA of more than 0.5 μm or 1.5 kb have been identified using mica-press-adsorption for electron microscopy[29]. Although spcDNA have long been suggested to be associated with genomic

Table 1
Four main types of eccDNAs and methods of detection.

Types	First discovered	Size	Properties	Methods of detection	Refs
spcDNA	1967	500bp-10kb (occasionally >10kb)	Resistant to denaturation, enhanced sedimentation velocity in neutral and alkaline solution, enhanced buoyant density in alkaline solution	**Isolation:** • Buoyant-density method, e.g., CsCl containing ethidium bromide followed by centrifugation • Rolling circle amplification (RCA), e.g., Circle-Seq, Mobilome-Seq, CIDER-Seq • CRISPR-CATCH	[18–20,23,27–30, 33–38]
t-circle	1995	Multiple units of 738bp	Highly supertwisted	**Library types:** • WGS • ATAC	
microDNA	2012	80-2000bp (>50% in 200-400bp)	Derived from nonrepetitive genomic sequences, are enriched in 5'-UTR of genes, exons, and CpG islands	**Imaging:** • Giemsa, acetoorcein, Feulgen, and DAPI staining • FISH • Electron microscopy	
ecDNA	1965 (as DM)	10^4-10^7 bp	Exists as pairs (DM) and singletons	**Image processing:** • ECdetect • EcSeg	

instability, their varied size and sequence content implies potentially different mechanisms of generation[17]. For example, preferential formation of spcDNA from Alu-rich regions in HeLa cells may be attributed to the juxtaposition of poly(A) sequence at the 5′ and the 3′ end of the Alu element[30]. Alternatively, spcDNA circularization could also result from the recombination mechanism. Homologous intrachromosomal recombination was proposed by Jones and Potter to explain the 9 bp direct repeats that occur in spcDNA[31]. Repeated circularization of multiple recombination events in the V_α and J_α regions of T-cell receptor α-chain could also explain the high copy number of spcDNA[32]. Whilst spcDNA are generated through hitherto unknown processes, mechanisms may exist that enable elements to loop out of the chromosomes and promote the joining of flanking DNA by illegitimate recombination[30].

1.1.2. Telomeric circles (t-circles)

In 1995, Nosek *et al.* reported the discovery of inverted terminal repeats that were made up of tandemly repeating units in yeast type 2 linear mitochondrial genomes (mtDNA)[33]. Taking *Candida parapsilosis* as an example, the terminus is comprised of a 738 bp repeating unit, with a 5′ single-stranded extension of about 110 nucleotides that is accessible to the enzymes. Later, Tomaska *et al.* used electrophoresis and electron microscope to reveal that the super-twisted circular conformation of these extragenomic molecules was derived from mitochondrial telomere repeats[34]. Although minicircular structures in mitochondrial DNA have been reported in several phylogenetically distinct species[39–43], their precise mechanism of generation and functions remains unexplored. While t-circles could be generated either by intramolecular recombination within the telomeric array or via telomeric loop extrusion, it was believed that t-circles maintain telomeric arrays of linear DNA through recombination. As DNA ends need to be protected against nucleolytic attacks and improper DNA metabolism, Tomaska *et al.* suggested that the various types of t-elements represent alternative strategies to tackle these obstacles[44]. For example, t-circles are believed to maintain telomeres integrity through alternative lengthening of telomeres (ALT) in 15 % of telomerase-negative cancers[45–47], thereby providing an alternative strategy for aberrant upregulation of cancer cell activity.

1.1.3. MicroDNA

A new form of eccDNA – microDNA was first found in 2012 in various tissues and cell lines[35]. Spanning 200 to 400 base pair long, these microDNA are enriched in 5′ UTR, exons, and CpG islands, and have a short region of micro-homology at the beginning and the end of the circles, suggesting the likelihood of microdeletions from the source genomic loci[35]. Unlike spcDNA, which typically span a few kilobase pairs and seem to originate from repetitive regions[7–9,48–50], microDNA originate from non-repetitive sequences, preferentially from high gene density areas. In 2015, Dillon *et al.* revealed microDNA were originated from DNA breaks or replication slippage following mismatch repair and loop excision[51]. The same study upon profiling microDNA from chicken DT40 cell lines lacking various crucial DNA repair proteins involved in non-homologous end joining (NHEJ), homologous recombination (HR), and microhomology-mediated end-joining (MMEJ), observed that microDNA were produced by all mutant strains, confirming that no single DNA repair pathways was responsible for generating microDNA[51]. In addition to these three potential mechanisms, Dillon *et al.* also pointed out these extra copies of genomic regions could alter cellular functions by protein titration and abnormal short RNAs production. More recently, using synthesized microDNA that resembled known microDNA regions, Paulsen *et al.* showed that microDNA express functional small regulatory RNA that are subsequently processed into mature microRNA (miRNA) and repressed endogenous targets[52]. Since microDNA that carry miRNA genes are functional, and miRNAs in turn are indispensable to animal development, cell differentiation and homeostasis[53], and tumor progression[54], it is likely that microDNA are key regulators of biological processes.

1.1.4. Extrachromosomal DNA (ecDNA)

In 2017, Turner *et al.* introduced yet another new type of eccDNA – ecDNA, a mega base pair amplified circular DNA that is visible in optical microscopy[19]. Accordingly, multiple image-based analysis tools were developed to identify ecDNA from DAPI-stained metaphases[19,38]. Unlike other eccDNA, ecDNA are almost never found in normal cells, but are large enough to carry driver oncogenes[19]. A subsequent study using AmpliconArchitect found that oncogenes amplified on ecDNA had higher transcripts compared to when the same genes were not amplified on ecDNA, even after normalization of their copy numbers, suggesting alterations to the genetic structure, such as enhanced chromatin accessibility[55]. Although the underlying mechanisms of ecDNA biogenesis are not yet fully elucidated, four models have been proposed, including breakage-fusion-bridge cycle, translocation-excision-deletion-amplification, episome, and chromothripsis. Details of these four models are further examined in the next section.

1.1.5. Breakage-fusion-bridge cycle

This concept was first introduced in 1951 by McClintock while studying the mechanisms responsible for mutable loci in maize [56]. The breakage-fusion-bridge cycle is initiated when newly broken ends of chromosomes at a meiotic mitosis cause the fusion between sister chromatids (Fig. 1**A**), resulting in a bridge configuration followed by separation of centromeres of the dicentric chromatid[56]. Because the break could occur anywhere between the two centromeres and the cycle continues in successive mitoses during development, breakage-fusion-bridge cycle results in extensive DNA ladder-like focal amplifications and large deletions, and potentially the formation of double minutes (DM)[57–59], a term often used in early studies to describe extrachromosomal structures.

1.1.6. Translocation-excision-deletion-amplification

Translocation and amplification are two important cytogenetic categories associated with tumor etiology[60]. The chromosomal translocations studied in various cancers suggested two routes to activate oncogenes: the activation of a proto-oncogene juxtaposed to a T-cell receptor gene or an immunoglobulin protein, and the creation of a fusion gene by the breaks of two coding regions [61]. DNA amplification is a frequent genetic abnormality in tumors, which are manifested as DM and homogenously staining regions (HSR) as cytogenetic hallmarks[62]. In some cases, translocation could concert with amplification to promote tumorigenesis. In 1996, Barr *et al.* employed FISH, RT-PCR and Southern blot to show the amplification of PAX3-FKHR or PAX7-FKHR fusion genes in 20 % of fusion-positive alveolar rhabdomyosarcomas[60], substantiating a sequential process through which oncogenes were activated. Another study unraveled the mechanism of non-syntenic co-amplification of MYC and ATBF1 in a neuroblastoma cell line, which involved multiple double-stranded breaks accompanied by a reciprocal t(8;16) translocation and deletion near the breakpoints[63]. In line with other findings[64,65], extra replication or loop formation could result in a DM configuration (Fig. 1**B**).

1.1.7. Episome

The concept of episome was first introduced in 1987 when Carroll *et al.* found a subclone of T5 transformant gave rise to a CAD episome containing donated CAD genes[66]. Gel electrophoresis showed these extrachromosomal molecules were 250 to 300 kilo-

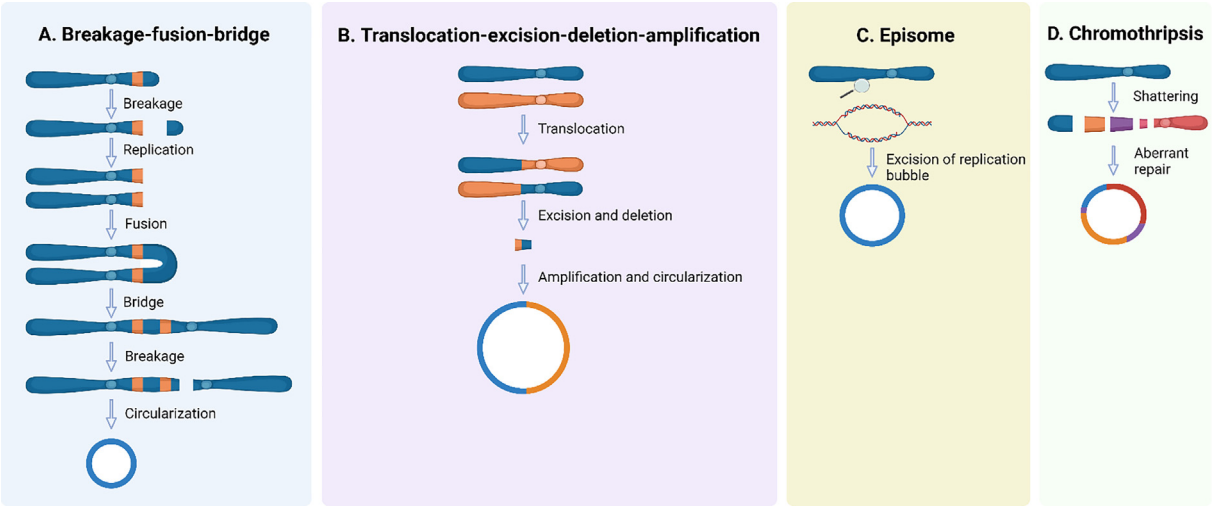

Fig. 1. Models of ecDNA generation. A. Breakage-fusion-bridge cycle, B. Translocation-excision-deletion-amplification, C. Episome, and D. Chromothripsis.

base pairs in size and were covalently closed. Like DM, these circular elements contain a functional origin of DNA replication and can replicate autonomously. To explore the episomal formation mechanism, Carroll *et al.* grew CAD episome containing T5 cells under nonselective conditions and found that the loss of episome was correlated to the loss of donated CAD genes, suggesting the formation of episome by corresponding chromosomal region deletion [67]. Two mechanisms were speculated by the authors, re-replication model and recombination across the looped replication domains[67]. While the prediction of the first model implies a rereplicated chromatid strand forming a "loop" structure, the latter model involves recombination of donated chromosomal sequences of sufficient size bearing origins of replication[68]. Another study on MYC carrying a DM has shown the amplified region was deleted at 8q24 in 68 % of the cases, which favored the episome model[69]. Interestingly, breakage across replication bubbles at stalled forks could also result in ecDNA formation (Fig. 1**C**)[70,71]. Consequently, episomal amplification could result in palindromic amplicons[71], or tyrosine kinase activation by the fusion between NUP214 and ABL1, promoting the pathogenesis of T-cell acute lymphoblastic leukemia (T-ALL)[72].

1.1.8. Chromothripsis

A remarkable phenomenon whereby extensive genomic rearrangements occur in a single catastrophic event was termed as chromothripsis by Stephens *et al.* in 2011[73]. Although the prevailing cancer evolution dogma indicated gradual acquisition of driver mutations, which resulted in increasing malignancy[74], somatic mutation outbursts might be a one-time event which promotes cancer development[73]. Importantly, chromothripsis could facilitate ecDNA generation (Fig. 1**D**)[73,75,76]. For example, the MYC containing DM was found to be generated by the shattering of chromosome 8 in a small cell lung cancer cell line[73]. While the mechanism for chromothripsis is unknown, a recent study showed that the process was dependent on poly(ADP-ribose) polymerases (PARP) and DNA-dependent protein kinase (DNA-PKcs) [77]. An error resulting in chromosome mis-segregation or intact chromatin bridge during the interphase could also pulverize the chromosomes[78], causing the scars in genome and DNA rearrangements, usually resulting in DNA circularization.

2. Functional characteristics of eccDNA

The diverse molecular and physiological functions of EccDNA summarized in Fig. 2 are described in detail this section.

2.1. Spatiotemporal dynamics of eccDNA

Antibiotic resistance (AbR) genes reside on mobile genetic elements (MGEs) such as plasmids, integrative and conjugative elements (ICEs), and various transposons[79]. Like plasmid DNA, eccDNAs carry critical genes that offer selective advantages in varying selective pressures (reviewed below in the section **Adaptation of eccDNAs under therapeutic response**). Since MGEs are mobile through various mechanisms, it is likely that eccDNAs are locomotive[80].

One aspect of eccDNA motility is its elimination by micronucleation[81–83], which was exemplified by the spontaneous extrusion of supernumerary MYCN amplified eccDNA into micronuclei [82]. It was shown that in a small percentage of cells, hybridization signals distributed peculiarly in clusters, adhered to nuclear membrane, and aggregated in nuclear protrusions[82,84], suggesting a spontaneous elimination process. The idea of an eccDNA hub was confirmed in a later study[85] where the authors labelled MYC ecDNAs with TetR-eGFP/TetR-eGFP(A206K) in COLO320-DM cells. Interestingly, treatment with 500 nM of a bromodomain and extraterminal (BET) protein inhibitor, JQ1, dispersed eccDNA hubs in COLO320-DM cells but did not alter the signal distribution of MYC in COLO320-HSR cells, implying the involvement of BET in hub maintenance[85]. Subsequently, Yi *et al.* established a CRISPR-based tracking technique which utilized sequences covering eccDNA-specific breakpoints to uncover disjointed eccDNA inheritance pattern during mitosis[86]. The authors found that the fluorescent signal was diluted during cellular cytoplasmic division and was reestablished once the two daughter cells entered the interphase; thereby providing direct visual evidence of the spatiotemporal dynamic feature of eccDNA[86].

Another aspect of eccDNA mobility is exemplified by the HIV-1 DNA integration into and disintegration from the host genome. 1-long terminal repeat (1-LTR) and 2-LTR circles are the two types of episomal HIV-1 DNAs that are particularly found in acutely infected cells such as the effector memory $CD4^+$ T cells[87]. Although these elements contain sequences for viral replication, their functions remain unknown and were considered as by-products of the reverse transcription. A recent study suggested that 2-LTR circles could serve as reservoirs for proviral integration due to their palindromic junctions being recognized by integrase. Interestingly, the cleavage was specific and could be improved by the integrase cofactor LEDGF/p75[88]. It is likely that 2-LTR could serve as a main source of substrate for integration when its number surpasses that of linear DNA. For example, in the presence of

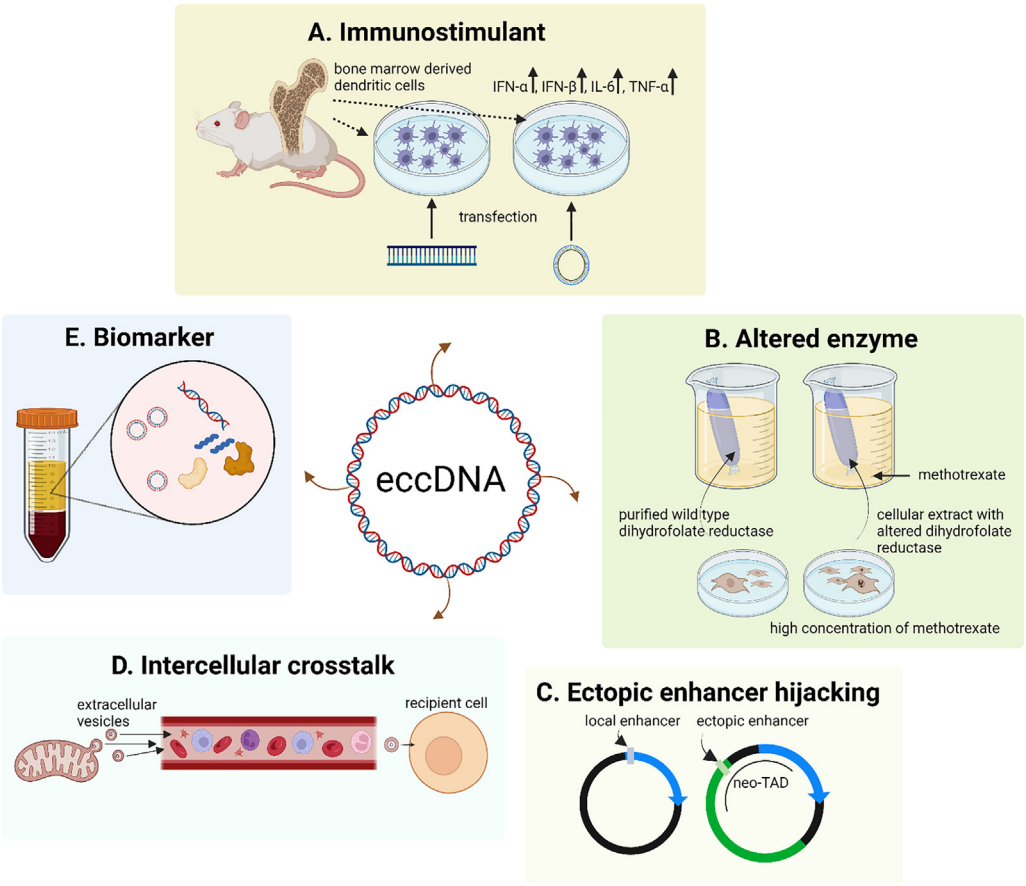

Fig. 2. Functional characteristics of eccDNAs. A. EccDNAs may function as innate immunostimulants that induce cytokine production. B. Cells carrying DMs at high concentration of methotrexate harbor altered dihydrofolate reductase enzyme, which has significant reduction of binding affinity for methotrexate, as measured by equilibrium dialysis. C. The complexity of eccDNA structure is exemplified by the hijacking of ectopic enhancer when the local enhancer is lost. A neoTAD could be formed by rearrangement during the process. D. EccDNAs could potentially mediate intercellular crosstalk. E. EccDNAs have been suggested by a few studies as biomarkers to progress disease surveillance, given that they are resistant to exonuclease and ribonuclease.

a HIV integrase inhibitor raltegravir[88]. In a recent study, clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) was used to excised HIV proviral DNA in NL4-3/Luc-transduced 293 T cells[89]. Upon ablation, circular DNA with full-length LTRs formed via intermolecular, and sense-sense joining could be detected for up to 14 days. These concatemers upregulated integrase and p24 production upon pTat and pRev cotransfection, suggesting that they could be transcriptionally active[89].

Like retroviruses, retrotransposons also involve reverse transcribed eccDNAs as part of their lifecycles[90]; therefore, eccDNAs could potentially characterize the reservoir of active transposons [13,36,90,91]. Interestingly, transposon display revealed integration of *ONSEN* transposons into the genome in *Arabidopsis* under heat stress and drugs, suggesting eccDNAs may contribute to genome evolution[90].

2.2. EccDNA as a mobile regulatory element

Due to the absence of centromeres, eccDNA are subjected to loss during nuclear envelope break down. However, studies revealed that eccDNA were tethered to chromosomes, which enabled acentric eccDNA to be efficiently passed onto daughter cells during mitosis[92–94]. Interestingly, eccDNA were associated with the periphery of prometaphase chromosome rosettes and were localized far away from the spindle poles, suggesting their dependence on antipolar forces[93]. Although the mechanisms of eccDNA-chromosomal adherence remains unclear, a study on the interaction between the origin of plasmid replication (oriP) and the viral protein EBNA-1 in Epstein-Barr virus (EBV) offered an unique insight[95]. The trans-activator protein EBNA-1 interacted with oriP and was thought to facilitate the anchorage of viral genomes on cellular chromosomes[96]. Analogously, Baiker *et al.* have shown the interaction between the origin of replication in simian virus 40 (SV40) genome, which attached to scaffold/matrix attachment region (S/MAR), and the chromosome scaffold, providing a mechanistic explanation of episomal stability and retention[97].

Although the idea that eccDNA might interact with chromosomes or with each other is not new[17,98], it was speculative and no concrete evidence was revealed. Recently, a model proposed ecDNA functioned as mobile regulatory elements that promoted the activity of chromosomal genes[99]. In this study, the authors performed Hi-C and RNAPII-associated ChIA-PET analysis on multiple GBM-patient-derived neurospheres and found ecDNA broadly contact the whole genome. The enrichment of trans-chromosomal interaction frequencies (nTIF) compared to the average genome-wide nTIF at 50 kb resolution was still significant after adjusting for the copy number. Furthermore, by comparing Histone H3 Lysine 27 acetyl (H3K27ac) peaks detected in the interacting loci on the ecDNAs, their chromosomal partners, and the genome-wide regions not contacting with ecDNAs, the authors showed ecDNA-chromosome interactions were associated with transcriptionally active sites[99]. Multiple lines of evidence have also corroborated the ecDNA-chromosomal interactome including: the significantly higher RNA transcription of chromosomal genes contacting ecDNAs, multi-color FISH validation, the higher interac-

tion frequencies mediated from ecDNA by comparing adjusted nTIF of subsampled regions on ecDNA and chromosomes with matched H3K27ac fold enrichment, the higher RNA expression level of genes connecting with ecDNA but with comparable RNAPII binding enrichment than those without connections. Although the dynamics of eccDNA diffusion and the stability of *trans*-interaction remain unknown, ecDNA could potentially act as mobile enhancers (Fig. 3) that greatly expand the transcriptional plasticity of a cell population[100].

2.3. Epigenetic landscape of eccDNAs

Although oncogene bearing eccDNAs could facilitate gene overexpression by merely increasing their copy number[19], the amount of DNA template was not the only factor that contributed to gene transcription[55 101 102,103]. Wu *et al.* integrated ATAC-seq profile with WGS data and found that the ATAC-seq signal was significantly higher in circular amplicons even after normalization of the DNA copy number[55]. In addition to the less compacted nucleosomal organization, co-amplification of the proximal enhancer and hijacking of the ectopic enhancer into highly rearranged MYCN amplified ecDNA have also been reported[101]. This finding was in line with an earlier study that found significant co-amplification of non-coding DNA beyond amplified oncogenes on ecDNA across several tumor types[102]. Interestingly, a recent study showed guide RNAs targeting an intergenic region near MYC- amplified ecDNAs significantly impaired cell growth[104]. Together these studies highlight new mechanisms by which eccDNA contribute to cancer progression.

2.4. EccDNA association with immune response

A recent study suggested that eccDNAs could function as potential innate immunostimulants in a manner which was dependent on the circular structure but not the underlying sequence[105]. The authors generated bone marrow-derived dendritic cells and bone marrow-derived macrophages, and compared their immune response (i.e. production of IFNα, IFNβ, IL-6, and TNF) between linear genomic DNA, eccDNA, and poly(dG:dC). Surprisingly, all aforementioned cytokines were significantly generated by eccDNA compared to linear DNA at varying concentrations. Similarly, these cytokines were significantly upregulated by eccDNA compared to poly(dG:dC) at lower concentrations, implicating the potency of the circular DNA on immune response. Furthermore, generation of linearized eccDNA by introducing one nick per circular DNA revealed that these linearized eccDNA behaved like linear DNAs and failed to activate cytokines; thus supporting the strong immunostimulant activity of eccDNA[105] and their potential induction of primary B cell and T helper type 2 responses[106].

Circularization of viral DNA by NHEJ pathway, on the other hand, has been suggested to alleviate the apoptotic effect in retroviral infected cells, albeit without excluding indirect mechanisms

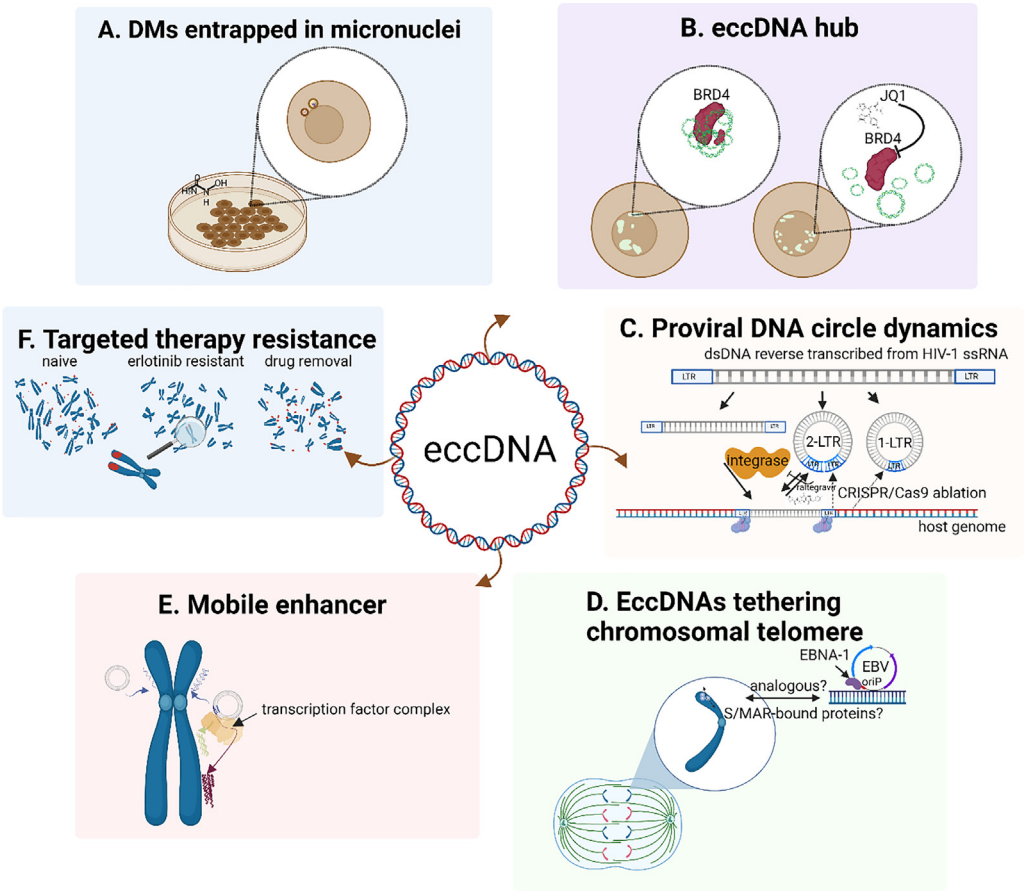

Fig. 3. EccDNAs are mobile. A. DMs entrapped in micronuclei could be eliminated upon hydroxyurea treatment, suggesting opportunities to improve chemotherapeutic regimen. B. Clusters of eccDNAs could form a hub which promotes intermolecular interactions. BET protein such as BRD4 facilitates such interaction, which could be disrupted by the BET inhibitor JQ1. C. Proviral DNA circles could be used as substrates for host genome integration. Upon CRISPR/Cas9 ablation, DNA circles with LTR are accumulated. D. EccDNAs are associated with the telomeric regions of chromosome rosettes at the onset of anaphase, and the tethering process may mimic viral vector behavior. E. EccDNAs could function as mobile enhancers that globally amplify chromosomal transcription. On the contrary, synthetic eccDNAs not carrying enhancer regions do not increase genome-wide RNA expression. F. The loss of EGFRvIII carrying DMs are associated with EGFR TKI resistance, suggesting eccDNAs' adaptation under environmental pressure.

for suppression[107]. Although unintegrated viral DNA expression could not downregulate human leukocyte antigen complex on resting $CD4^+$ T cells, it sensitized infected cells to be targeted and killed by functional cytotoxic T cells[108]. Moreover, episomal HIV-1 DNA has been shown to be transcriptionally active, and could generate functional viral proteins such as Tat and Nef[109–112], which may contribute to the CC-chemokine production in infected macrophages and the recruitment of lymphocytes[113].

2.5. Adaptation of eccDNAs under therapeutic response

The capability of eccDNAs to dynamically regulate themselves under environmental pressures is well known. In 1979, Kaufman *et al.* observed the unstably amplified dihydrofolate reductase gene (DHFR) on DM in higher concentrations of methotrexate[114]. As DHFR converts dihydrofolic acid to tetrahydrofolic acid, which is indispensable for synthesizing purines and pyrimidines, the presence of DHFR-carrying DM may partly explain methotrexate resistance. Similarly, later studies also identified an association between amplified DHFR and small circular DM in various cell types[104,115–117]. Surprisingly, DHFR was altered spontaneously and exhibited a 270-fold reduction in binding affinity for methotrexate[115], which further contributed to the inhibitor insensitivity by reducing the enzymatic activity. When methotrexate was absent, the growth rate of methotrexate-resistant murine S-180 cell was inversely correlated to the copy number of DHFR carrying DM[118], implying that the cells lacking DHFR amplification had a growth advantage when the drug was removed, and were likely selected during uneven segregation. Interestingly, the resistant S-180 line lost most of its DM after continued selection in methotrexate-containing medium and acquired DHFR genes on a few chromosomes[118]. This phenomenon was also reported in other studies[119–121], which could be revealed by Giemsa-banding.

In addition to the association between DHFR carrying eccDNA and antifolate resistance, the loss of oncogene bearing eccDNA was also correlated with the substantial reduction of tumorigenicity in several human tumor cell lines[81]. Von Hoff *et al.* showed that the treatment of a low concentration of hydroxyurea on HL60, COLO 320, NB4, and SF188 cells promoted the loss of MYC amplified DM, which is involved in the entrapment of DM within the micronuclei[81]. In a clinical trial conducted in 2001, researchers investigated whether a low dosage of hydroxyurea could downgrade DMs in 16 patients with advanced ovarian carcinomas[122]. Results revealed that 45 % of the patients showed more than 50 % reduction of the number of spreads with DM containing tumor cells, while one patient demonstrated 52 % reduction of the c-myc copy number after hydroxyurea treatment[122]. Similar mechanism of eccDNA extrusion was also proposed in another study[123], where 2 Gy fractions up to a total radiation dose of 28 Gy resulted in the reduction of MDR1 and MYCC bearing eccDNA via entrapment in micronuclei in multidrug-resistant lines.

Other chemotherapeutic agents have also been reported to regulate gene amplifications on eccDNAs. For example, lower levels of mitoxantrone induced ABCG2 amplification via DM in the SF295 glioblastoma cell line[124]. Gemcitabine at a 7500X lower concentration of hydroxyurea effectively reduced DM in an ovarian cancer cell line by incorporating the amplicons and γ-H2AX signals into micronuclei[125]. The sensitivity of cisplatin induced apoptosis could be reversed by introducing antisense oligonucleotides targeting against MDM2 mRNA in human glioblastoma cells[126]. As the development of resistance to chemotherapeutic drugs is considered as one of the reasons for the failure of cancer treatment, and both DM and HSR were shown as cytogenetic manifestations in cancer cells, studying the role of eccDNAs in chemotherapeutic drug response may offer mechanistic insights into acquired resistance.

Targeted cancer therapies that interfere with tumor cell growth by interacting with specific molecules have shown some promising results as evident from the high response rates demonstrated by patients on these regimens. However, the prolonged benefit of majority of these therapies are limited by the eventual development of resistance of the tumor cells[127–129]. Nathanson *et al.* have found that resistance to erlotinib, an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), in glioblastoma was conferred by the reversible loss of eccDNAs containing the active oncogenic variant EGFRvIII, which bestowed an optimal cellular state for growth and survival[130]. This finding was later recapitulated in another study[104]. Interestingly, $EGFR^+$ HSRs were observed throughout the entire stages of naïve, drug resistant, and drug retraction, whereas $EGFR^+$ eccDNAs were only completely lost in erlotinib-resistant GBM cells, suggesting an adaptive route by which tumors can evade targeted therapy. Similarly, Song *et al.* recently showed that BRAF amplification also causes challenges to targeted therapy dosages[131]. The authors developed a melanoma model of dual MAPK inhibitor (MAPKi; specifically, vemurafenib [BRAFi] and selumetinib [MEKi]) resistance that bore $BRAF^{V600}$ amplifications either through DM or HSR. They found that drug-resistant plasticity was coupled with focal amplifications, and that inconstant drug dosage prevented the switch from DM to HSR. Moreover, a different form of cell death, ferroptosis, occurred during BRAF amplification mediated MAPKi resistance, extending the resistant mechanisms beyond cellular dedifferentiation[131].

2.6. EccDNA confer resistance to herbicide

EccDNA dynamics has also been suggested to promote plant evolution. Koo *et al.* reported that eccDNA based EPSPS amplification was associated with rapid glyphosate resistance in the crop weed *Amaranthus palmeri* through adaptive evolution[132]. Interestingly, a sexual transmission study that crossed a female *A. palmeri* lacking eccDNA and a male *A. palmeri* carrying eccDNA showed positive signals associated with mitotic metaphase chromosomes in the descendants, indicating the successful transmission of herbicide resistance to the offspring via eccDNA.

2.7. EccDNA and aging

Recently, Hull *et al.* demonstrated the transcription of tandem CUP1 copies stimulated CUP1 encoding eccDNA in yeast that were aged under environmental exposure to copper[133], a process triggered by factors such as Sae2, Mre11 and Mus81 that are involved in DNA repair. In addition to facilitating adaptive evolution, eccDNAs accumulation in the nuclei may also promote ageing and tumorigenesis. Current hypothesis suggests the transport of damaged DNA via eccDNA out from the nucleus into the cytosol, where a cell-autonomous nucleic acids (NA) degradation machinery is triggered to keep NA below the immunostimulatory threshold [134]. However, during ageing, the increase of dysfunctional nuclear pore complexes (NPCs) may result in the accretion of eccDNA in the nucleus[135]. The consequent accumulation of DNA damage in turn promotes cellular senescence and apoptosis [136], further indicating a strong link between eccDNAs and ageing.

2.8. EccDNAs may potentiate intercellular crosstalk

Circular RNAs (circRNAs), recently found to be amply supplied and stable in exosomes[137], were suggested to mediate intercellular crosstalk in the tumor microenvironment by involving cancer and stromal cells[138]. For example, hepatocellular carcinoma

(HCC) cells with high metastatic capability could transfer their metastatic potentiality to other HCC cells with low or no metastatic capability by secreting exosomes with circPTGR1[139], exosomal circ-CCAC1 in cholangiocarcinoma was transmitted to endothelial cells, which promoted angiogenesis by downregulating junctional proteins[140]. Similarly, could eccDNA trapped micronuclei represent an entity facilitating intercellular network? Despite extracellular micronuclei with DM being reported in some studies[83,141,142], which suggested micronuclei could be expelled from the cell and serve as a repertoire of DNA elements, the impact on intercellular genetic communication remains unclear. Interestingly, the content of micronuclei could be shuttled into multivesicular bodies via direct contact[143]. Nevertheless, mitochondrial circular genome could not only be transferred through direct cell–cell contact[144,145], but also via circulating extracellular vesicles[146], suggesting circular DNAs as potent paracrine/endocrine signaling factors. However, as studies on eccDNAs as communicators between cells are limited and still speculative, additional are needed to determine this potential role of eccDNAs.

2.9. EccDNAs as potential biomarkers

Episomal HIV-1 DNAs were found in patients with advanced central nervous system damage[147], and patients on antiretroviral therapy (ART)[148–150]. Although the roles of these episomes are still debatable, a study comparing the envelop sequences in episomal and proviral genomes before viral rebound upon treatment interruption with those in emergent viral RNA[150] suggested that episomal HIV-1 could fuel viremia rebound. Moreover, episomal HIV-1 genomes could be used as a marker to monitor ART, due to its lability *in vivo*, and given that traditional methods were not sensitive in viral reservoirs detection[151].

A recent study using four paired primary and metastatic tissues of high grade serous ovarian cancer (HGSOC) highlighted the association between DNMT1$^{\text{circle10302690-10302961}}$ downregulation and HGSOC metastasis[152], suggesting certain eccDNA element could be considered as a prognostic marker.

Liquid biopsy, which includes cell-free DNA (cfDNA), circulating tumor cells and exosomes, has made great progress in recent years due to its non-invasiveness and informativeness[153]. EccDNAs are a type of cfDNA in the circulating system and evidence of their role in disease association and progress surveillance suggests their potential to be harnessed as biomarkers. For example, a recent study comparing eccDNAs from the plasma of 6 lung adenocarcinoma (LUAD) and 10 healthy individuals reported of a higher frequency of nine top ranked eccDNAs in LUAD samples when compared to the healthy group. Interestingly, the study also found that DOCK1, PPIC, TBC1D16, and RP11-370A5.1 were uniquely encoded in eccDNAs in LUAD group[154]. Similarly, another study showed that the cell-free microDNA present in tumor lung tissue specimens were longer than those in paired normal lung samples; moreover, serum and plasma samples collected prior to surgery were enriched with longer microDNA compared with that obtained from the same patients following surgical tumor resection[15]. Interestingly, the formation of eccDNAs was found to be dependent on the lineage of cancer[51].

Although unique eccDNAs were able to be identified in some diseases, characteristics such as high GC content, repetitiveness, and low quantity in plasma[154] may hamper their application in clinical setting by increasing the difficulty in primer design and temperature control. While common eccDNA detection methods involves RCA, which is an efficient isothermal DNA amplification procedure, the synthesis cost is high, and primers are still in need. Nevertheless, a recent study using a label-free fluorescent biosensor to detect circRNA provides an ultrasensitive alternative to identify eccDNA[155].

While circRNA was reported to degrade completely within 15 s in 25 % serum[156], the average half-life of fetal eccDNA in the maternal blood was found to be 29.7 min[157]. The half-lives of extracellular microRNAs (ex-miRNAs), on the other hand, varied between ex-miRNA entities and the species[158,159]. It is important to note that while the stability of ex-miRNAs were measured in cell culture [158,159], fetal eccDNA kinetics were determined from blood samples collected from pregnant women before delivery and at multiple time points postpartum[157]. Serial time point collection of blood was important as it correctly reflects the half-lives of eccDNA in the biological system. While long non-coding RNAs (lncRNAs) have drawn attention as molecular biomarker for cancer prognosis[160], their rate of decay has also only been measured in *in vitro* models[161,162]. While the half-lives of circRNA, eccDNA, ex-miRNA, lncRNA are yet to be directly compared from genome-wide analyses, based on eccDNAs' size distribution, sequence, resistance to exonuclease or ribonuclease, and their stability compared to RNA, growing evidence indicates that eccDNAs could serve as potent biomarkers for disease surveillance.

2.10. EccDNA databases

To date, three eccDNA databases have been introduced (Fig. 4) [163–165], which compiled eccDNAs from different resources and are focused on diverse aspects of eccDNAs. While CircleBase,

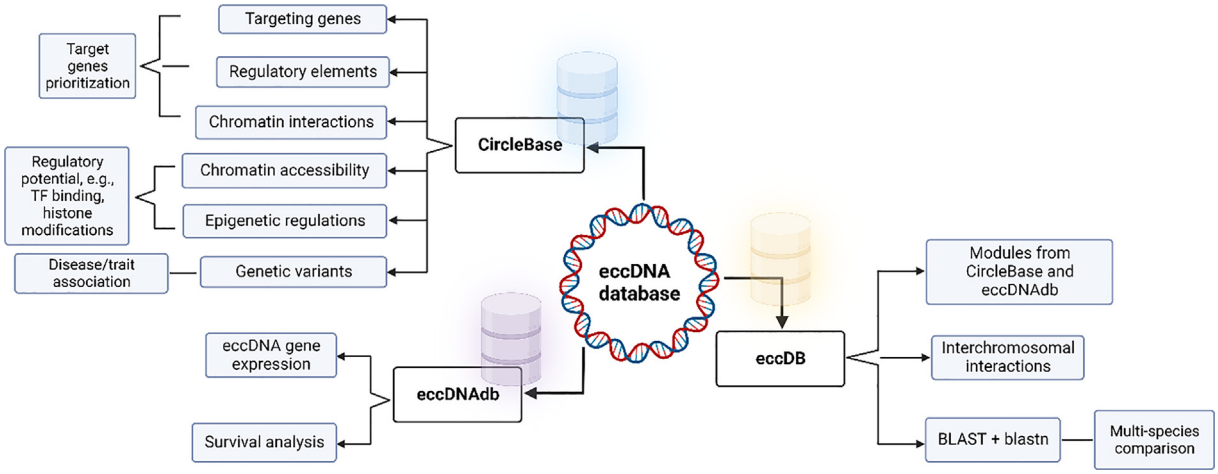

Fig. 4. EccDNA databases and their associated functional annotations.

the first integrated platform for eccDNA functional interpretation, comprises 601,036 eccDNAs collected from 13 papers[163], both eccDNAdb[164] and eccDB[165] profiled eccDNAs based on different library types and computational methods. To characterize potential eccDNA function, six modules comprising of various functional databases were used, and the diffusion algorithm PageRank was used to prioritize genes interacting with eccDNAs based on three of the modules[163]. While eccDNAdb focused on the prognostic value of eccDNA genes[164], eccDB complemented CircleBase database by incorporating eccDNA interchromosomal interactions and evolutionary relationships through multi-species sequence comparisons[165].

3. Summary and outlook

In this review, we summarize the current state of understanding of eccDNAs pertaining to their discovery, prevalence across multiple species and cancer types, classification and associated formation mechanisms, and physiological characteristics. Although the knowledge that eccDNAs contribute to cancer progression has been known for decades, gaps in our knowledge of eccDNAs remain and continue to be one of the intractable challenges faced by cancer researchers[166]. Although the overall frequency of DM in primary cancer has been reported to be 1.4 % based on the Mitelman database[167,168], a recent study suggests its frequency is far greater than previously inferred[19].

1. Future research on eccDNA integration and dissociation is needed. Since sequence and structure could contribute to the selective fragility of the genome[169,170], what are specific features of the boundaries. Are there specific chromatin organization or underlying DNA sequences?
2. While the prominent mechanism of how eccDNAs promote evolution of cancer cells relies on competitive advantage offered by uneven segregation of oncogene amplified eccDNAs during mitosis, the detailed molecular mechanisms are only now beginning to emerge and will require detailed mechanistic studies[84,104,171–173]. An important contributor to this puzzle could be that eccDNA can behave like enhancer elements and may traverse the nucleus to enable global chromosomal contacts thus introducing transcriptional plasticity in a cell population. Colocalization of eccDNAs which form a hub and recruit RNA polymerase not only provides a plausible mechanism of high oncogene transcriptional rate and intratumoral heterogeneity, but also offers promising avenues for the eccDNA hub directed cancer therapies.
3. Another important outstanding question is if DMs replicate independently. While some studies suggested DMs replicate synchronously with chromosomal in S phase[174,175], others suggested they were derived de novo from HSR fragmentation [176]. Nevertheless, the separation of DM's sister elements during G1 phase prevents the formation of quadruple chromatids [177], and likely alleviate their numerical heterogeneity between cells which results from their anomalous segregation during cell cycle. The mechanism of separation is unclear because the chromatin fiber organization of DMs is currently unknown[177]. Furthermore, DMs attach to nucleolar matter near the end of chromosome arm during metaphase[92], but what force drives scattered DMs inside the cytoplasm in prophase to relocate to the chromosome ends in metaphase?
4. Finally, aside from the elucidation of the eccDNA replication mechanism during cellular division, more studies on eccDNAs' roles in clonal dynamics, their targetable vulnerabilities, and their half-lives are needed to fully realize their translational value.

CRediT authorship contribution statement

Manrong Wu: Writing – original draft. **Kunal Rai:** Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Some elements of the **Figures** are from BioRender.com. The authors thank Dr. Uttara Saran, Dr. Christopher Terranova, and Yingda Jiang for corrections and suggestions. The authors would like to acknowledge financial support from National Institutes of Health (R01CA245395, R01CA226269, R01CA222214, R21CA256278).

References

[1] Navashin MS. Unbalanced somatic chromosomal variation in Crepis. University of California publications in agricultural sciences, 6. Berkeley: Cambridge University Press; 1930. p. 95–106.
[2] McClintock B. A correlation of ring-shaped chromosomes with variegation in Zea mays. PNAS 1932;18(12):677–81.
[3] Hotta Y, Bassel A. Molecular size and circularity of DNA in cells of mammals and higher plants. PNAS 1965;53(2):356–62.
[4] Stahl FW. Proc. Soc. de Chim. Phys, 11th Annual Reunion 1962:194.
[5] Cox D, Yuncken C, Spriggs AI. Minute chromatin bodies in malignant tumours of childhood. Lancet 1965;1(7402):55–8.
[6] Lubs HA, Salmon JH. The chromosomal complement of human solid tumors. II. Karyotypes of glial tumors. J Neurosurg 1965;22:160–8.
[7] Stanfield SW, Lengyel JA. Small circular DNA of Drosophila melanogaster: chromosomal homology and kinetic complexity. PNAS 1979;76(12):6142–6.
[8] Stanfield SW, Helinski DR. Cloning and characterization of small circular DNA from Chinese hamster ovary cells. Mol Cell Biol 1984;4(1):173–80.
[9] Sunnerhagen P, Sjöberg RM, Karlsson AL, Lundh L, Bjursell G. Molecular cloning and characterization of small polydisperse circular DNA from mouse 3T6 cells. Nucl Acids Res 1986;14(20):7823–38.
[10] Møller HD, Parsons L, Jørgensen TS, Botstein D, Regenberg B. Extrachromosomal circular DNA is common in yeast. PNAS 2015;112 (24):3114–22.
[11] Shoura MJ, Gabdank I, Hansen L, Merker J, Gotlib J, et al. Intricate and cell type-specific populations of endogenous circular DNA (eccDNA) in Caenorhabditis elegans and Homo sapiens. G3 (Bethesda) 2017;7 (10):3295–303.
[12] Møller HD, Ramos-Madrigal J, Prada-Luengo I, Gilbert MTP, Regenberg B. Near-random distribution of chromosome-derived circular DNA in the condensed genome of pigeons and the larger, more repeat-rich human genome. Genome Biol Evol 2020;12(1):3762–77.
[13] Wang K, Tian H, Wang L, Wang L, Tan Y, et al. Deciphering extrachromosomal circular DNA in Arabidopsis. Comput Struct Biotechnol J 2021;19:1176–83.
[14] Bailey C, Shoura MJ, Mischel PS, Swanton C. Extrachromosomal DNA – relieving heredity constraints, accelerating tumour evolution. Ann Oncol 2020;31(7):884–93.
[15] Kumar P, Dillon LW, Shibata Y, Jazaeri AA, Jones DR, et al. Normal and cancerous tissues release extrachromosomal circular DNA (eccDNA) into the circulation. Mol Cancer Res 2017;15(9):1197–205.
[16] Schmidt H, Taubert H, Lange H, Kriese K, Schmitt WD, et al. Small polydispersed circular DNA contains strains of mobile genetic elements and occurs more frequently in permanent cell lines of malignant tumors than in normal lymphocytes. Oncol Rep 2009;22(2):393–400.
[17] Cohen S, Regev A, Lavi S. Small polydispersed circular DNA (spcDNA) in human cells: association with genomic instability. Oncogene 1997;14 (8):977–85.
[18] Bafna V, Mischel PS. Extrachromosomal DNA in Cancer. Annu Rev Genomics Hum Genet 2022;23:29–52.
[19] Turner KM, Deshpande V, Beyter D, Koga T, Rusert J, et al. Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. Nature 2017;543:122–5.
[20] Kumar P, Kiran S, Saha S, Su Z, Paulsen T, et al. ATAC-seq identifies thousands of extrachromosomal circular DNA in cancer and cell lines. Sci Adv 2020;6 (20). https://doi.org/10.1126/sciadv.aba2489.
[21] Møller HD. Circle-Seq: isolation and sequencing of chromosome-derived circular DNA elements in cells. Methods Mol Biol 2020;**2119**:165–181.

[22] Koche RP, Rodriguez-Fos E, Helmsauer K, Burkert M, MacArthur IC, et al. Extrachromosomal circular DNA drives oncogenic genome remodeling in neuroblastoma. Nat Genet 2020;52:29–34.
[23] Hung KL, Luebeck J, Dehkordi SR, Coruh C, Law JA, et al. Targeted profiling of human extrachromosomal DNA by CRISPR-CATCH. bioRxiv 2021. https://doi.org/10.1101/2021.11.28.470285.
[24] Fan X, Yang C, Li W, Bai X, Zhou X, et al. SMOOTH-seq: single-cell genome sequencing of human cells on a third-generation sequencing platform. Genome Biol 2021;22:195.
[25] Paulsen T, Kumar P, Koseoglu MM, Dutta A. Discoveries of Extrachromosomal Circles of DNA in Normal and Tumor Cells. Trends Genet 2018;34(4):270–8.
[26] Liao Z, Jiang W, Ye L, Li T, Yu X, et al. Classification of extrachromosomal circular DNA with a focus on the role of extrachromosomal DNA (ecDNA) in tumor heterogeneity and progression. Biochim Biophys Acta Rev Cancer 2020;1874(1):188392.
[27] Radloff R, Bauer W, Vinograd J. A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in HeLa cells. PNAS 1967;57(5):1514–21.
[28] Smith CA, Vinograd J. Small polydisperse circular DNA of HeLa cells. J Mol Biol 1972;69(2):163–78.
[29] Kunisada T, Yamagishi H, Ogita Z, Kirakawa T, Mitsui Y. Appearance of extrachromosomal circular DNAs during in vivo and in vitro ageing of mammalian cells. Mech Ageing Dev 1985;29(1):89–99.
[30] Kunisada T, Yamagishi H. Sequence organization of repetitive sequences enriched in small polydisperse circular DNAs from HeLa cells. J Mol Biol 1987;198(4):557–65.
[31] Jones RS, Potter SS. LI sequences in HeLa extrachromosomal circular DNA: Evidence for circularization by homologous recombination. PNAS 1985;82 (7):1989–93.
[32] Fujimoto S, Yamagishi H. Isolation of an excision product of T-cell receptor alpha-chain gene rearrangements. Nature 1987;327(6119):242–3.
[33] Nosek J, Dinouël N, Kovac L, Fukuhara H. Linear mitochondrial DNAs from yeasts: telomeres with large tandem repetitions. Mol Gen Genet 1995;247 (1):61–72.
[34] Tomaska L, Nosek J, Makhov AM, Pastorakova A, Griffith JD. Extragenomic double-stranded DNA circles in yeast with linear mitochondrial genomes: potential involvement in telomere maintenance. Nucl Acids Res 2000;28 (22):4479–87.
[35] Shibata Y, Kumar P, Layer R, Willcox S, Gagan JR, et al. Extrachromosomal microDNAs and chromosomal microdeletions in normal tissues. Science 2012;336(6077):82–6.
[36] Lanciano S, Zhang P, Llauro C, Mirouze M. Identification of extrachromosomal circular forms of active transposable elements using Mobilome-Seq. Methods Mol Biol 2021;2250:87–93.
[37] Mehta D, Cornet L, Hirsch-Hoffmann M, Zaidi SS, Vanderschuren H. Full-length sequencing of circular DNA viruses and extrachromosomal circular DNA using CIDER-Seq. Nat Protoc 2020;15:1673–89.
[38] Rajkumar U, Turner K, Luebeck J, Deshpande V, Chandraker M, et al. EcSeg: semantic segmentation of metaphase images containing extrachromosomal DNA. iScience 2019;21:428–35.
[39] Shapiro TA, Englund PT. The structure and replication of kinetoplast DNA. Annu Rev Microbiol 1995;49:117–43.
[40] Palmer JD, Shields CR. Tripartite structure of the Brassica campestris mitochondrial genome. Nature 1984;307:437–40.
[41] Griffiths AJ. Fungal senescence. Annu Rev Genet 1992;26:351–72.
[42] Fauron C, Casper M, Gao Y, Moore B. The maize mitochondrial genome: dynamic, yet functional. Trends Genet 1995;11(6):228–35.
[43] Zhang Z, Green BR, Cavalier-Smith T. Single gene circles in dinoflagellate chloroplast genomes. Nature 1999;400(6740):155–9.
[44] Tomaska L, Nosek J, Kramara J, Griffith JD. Telomeric circles: universal players in telomere maintenance? Nat Struct Mol Biol 2009;16(10):1010–5.
[45] Cesare AJ, Reddel RR. Telomere uncapping and alternative lengthening of telomeres. Mech Ageing Dev 2008;129(1–2):99–108.
[46] Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer 1997;33(5):787–91.
[47] Tomaska L, McEachern MJ, Nosek J. Alternatives to telomerase: keeping linear chromosomes via telomeric circles. FEBS Lett 2004;567(1):142–6.
[48] Bertelsen AH, Humayun MZ, Karfopoulos SG, Rush MG. Molecular characterization of small polydisperse circular deoxyribonucleic acid from an African green monkey cell line. Biochemistry 1982;21(9):2076–85.
[49] Motejlek K, Schindler D, Assum G, Krone W. Increased amount and contour length distribution of small polydisperse circular DNA (spcDNA) in Fanconi anemia. Mutat Res 1993;293(3):205–14.
[50] Wang T, Zhang H, Zhou Y, Shi J. Extrachromosomal circular DNA: a new potential role in cancer progression. J Transl Med 2021;19:257.
[51] Dillon LW, Kumar P, Shibata Y, Wang YH, Willcox S, et al. Production of extrachromosomal MicroDNAs is linked to mismatch repair pathways and transcriptional activity. Cell Rep 2015;11(11):1749–59.
[52] Paulsen T, Shibata Y, Kumar P, Dillon L, Dutta A. Small extrachromosomal circular DNAs, microDNA, produce short regulatory RNAs that suppress gene expression independent of canonical promoters. Nucl Acids Res 2019;47 (9):4586–96.
[53] Gebert LFR, MacRae IJ. Regulation of microRNA function in animals. Nat Rev Mol Cell Biol 2019;20:21–37.
[54] Peng Y, Croce CM. The role of MicroRNAs in human cancer. Sig Transduct Target Ther 2016;1:15004.
[55] Wu S, Turner KM, Nguyen N, Raviram R, Erb M, et al. Circular ecDNA promotes accessible chromatin and high oncogene expression. Nature 2019;575:699–703.
[56] McClintock B. Chromosome organization and genic expression. Cold Spring Harb Symp Quant Biol 1951;16:13–47.
[57] Toledo F, Roscouet DL, Buttin G, Debatisse M. Co-amplified markers alternate in megabase long chromosomal inverted repeats and cluster independently in interphase nuclei at early steps of mammalian gene amplification. EMBO J 1992;11(7):2665–73.
[58] Singer MJ, Mesner LD, Friedman CL, Trask BJ, Hamlin JL. Amplification of the human dihydrofolate reductase gene via double minutes is initiated by chromosome breaks. PNAS 2000;97(14):7921–6.
[59] Nones K, Waddell N, Wayte N, Patch AM, Bailey P, et al. Genomic catastrophes frequently arise in esophageal adenocarcinoma and drive tumorigenesis. Nat Commun 2014;5:5224.
[60] Barr FG, Nauta LE, Davis RJ, Schäfer BW, Nycum LM, et al. In vivo amplification of the PAX3-FKHR and PAX7-FKHR fusion genes in alveolar rhabdomyosarcoma. Hum Mol Genet 1996;5(1):15–21.
[61] Rabbitts TH. Chromosomal translocations in human cancer. Nature 1994;372 (6502):143–9.
[62] Schwab M, Amler LC. Amplification of cellular oncogenes: a predictor of clinical outcome in human cancer. Genes Chrom Cancer 1990;1(3):181–93.
[63] Van Roy N, Vandesompele J, Menten B, Nilsson H, De Smet E, et al. Translocation-excision-deletion-amplification mechanism leading to nonsyntenic coamplification of MYC and ATBF1. Genes Chromosomes Cancer 2006;45(2):107–17.
[64] Storlazzi CT, Fioretos T, Paulsson K, Strömbeck B, Lassen C, et al. Identification of a commonly amplified 4.3 Mb region with overexpression of C8FW, but not MYC in MYC-containing double minutes in myeloid malignancies. Hum Mol Genet 2004;13(14):1479–85.
[65] Martín-Subero JI, Odero MD, Hernandez R, Cigudosa JC, Agirre X, et al. Amplification of IGH/MYC fusion in clinically aggressive IGH/BCL2-positive germinal center B-cell lymphomas. Genes Chromosomes Cancer 2005;43 (4):414–23.
[66] Carroll SM, Gaudray P, De Rose ML, Emery JF, Meinkoth JL, et al. Characterization of an episome produced in hamster cells that amplify a transfected CAD gene at high frequency: functional evidence for a mammalian replication origin. Mol Cell Biol 1987;7(5):1740–50.
[67] Carroll SM, DeRose ML, Gaudray P, Moore CM, Needham-Vandevanter DR, et al. Double minute chromosomes can be produced from precursors derived from a chromosomal deletion. Mol Cell Biol 1988;8(4):1525–33.
[68] Schimke RT, Sherwood SW, Hill AB, Johnston RN. Overreplication and recombination of DNA in higher eukaryotes: potential consequences and biological implications. PNAS 1986;83(7):2157–61.
[69] Storlazzi CT, Fioretos T, Surace C, Lonoce A, Mastrorilli A, et al. MYC-containing double minutes in hematologic malignancies: evidence in favor of the episome model and exclusion of MYC as the target gene. Hum Mol Genet 2006;15(6):933–42.
[70] Windle BE, Wahl GM. Molecular dissection of mammalian gene amplification: new mechanistic insights revealed by analyses of very early events. Mutat Res 1992;276(3):199–224.
[71] Vogt N, Lefèvre SH, Apiou F, Dutrillaux AM, Cör A, et al. Molecular structure of double-minute chromosomes bearing amplified copies of the epidermal growth factor receptor gene in gliomas. PNAS 2004;101(31):11368–73.
[72] Graux C, Cools J, Melotte C, Quentmeier H, Ferrando A, et al. Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. Nat Genet 2004;36(10):1084–9.
[73] Stephens PJ, Greenman CD, Fu B, Yang F, Bignell GR, et al. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. Cell 2011;144(1):27–40.
[74] Jones S, Chen WD, Parmigiani G, Diehl F, Beerenwinkel N, et al. Comparative lesion sequencing provides insights into tumor evolution. PNAS 2008;105 (11):4283–8.
[75] Rausch T, Jones DTW, Zapatka M, Stütz AM, Zichner T, et al. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Cell 2012;148(1–2):59–71.
[76] Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, et al. Chromothripsis from DNA damage in micronuclei. Nature 2015;522:179–84.
[77] Shoshani O, Brunner SF, Yaeger R, Ly P, Nechemia-Arbely Y, et al. Chromothripsis drives the evolution of gene amplification in cancer. Nature 2021;591(7848):137–41.
[78] Ly P, Cleveland DW. Rebuilding chromosomes after catastrophe: emerging mechanisms of chromothripsis. Trends Cell Biol 2017;27(12):917–30.
[79] Garcillán-Barcia MP, Alvarado A, de la Cruz F. Identification of bacterial plasmids based on mobility and plasmid population biology. FEMS Microbiol Rev 2011;35(5):936–56.
[80] van Leen E, Brückner L, Henssen AG. The genomic and spatial mobility of extrachromosomal DNA and its implications for cancer therapy. Nat Genet 2022;54:107–14.
[81] Von Hoff DD, McGill JR, Forseth BJ, Davidson KK, Bradley TP, et al. Elimination of extrachromosomally amplified MYC genes from human tumor cells reduces their tumorigenicity. PNAS 1992;89(17):8165–9.
[82] Ambros IM, Rumpler S, Luegmayr A, Hattinger CM, Strehl S, et al. Neuroblastoma cells can actively eliminate supernumerary MYCN gene copies by micronucleus formation–sign of tumour cell revertance? Eur J Cancer 1997;33(12):2043–9.

[83] Shimizu N, Itoh N, Utiyama H, Wahl GM. Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during S phase. J Cell Biol 1998;140(6):1307–20.
[84] Lundberg G, Rosengren AH, Håkanson U, Stewénius H, Jin Y, et al. Binomial mitotic segregation of MYCN-carrying double minutes in neuroblastoma illustrates the role of randomness in oncogene amplification. PLoS ONE 2008;3(8):e3099.
[85] Hung KL, Yost KE, Xie L, Shi Q, Helmsauer K, et al. ecDNA hubs drive cooperative intermolecular oncogene expression. Nature 2021;600 (7890):731–6.
[86] Yi E, Gujar AD, Guthrie M, Kim H, Zhao D, et al. Live-cell imaging shows uneven segregation of extrachromosomal DNA elements and transcriptionally active extrachromosomal DNA hubs in cancer. Cancer Discov 2022;12(2):468–83.
[87] Martinez-Picado J, Zurakowski R, Buzón MJ, Stevenson M. Episomal HIV-1 DNA and its relationship to other markers of HIV-1 persistence. Retrovirology 2018;15:15.
[88] Richetta C, Thierry S, Thierry E, Lesbats P, Lapaillerie D, et al. Two-long terminal repeat (LTR) DNA circles are a substrate for HIV-1 integrase. J Biol Chem 2019;294(20):8286–95.
[89] Lai M, Maori E, Quaranta P, Matteoli G, Maggi F, et al. CRISPR/Cas9 ablation of integrated HIV-1 accumulates proviral DNA circles with reformed long terminal repeats. J Virol 2021;95(23):e0135821.
[90] Thieme M, Lanciano S, Balzergue S, Daccord N, Mirouze M, et al. Inhibition of RNA polymerase II allows controlled mobilisation of retrotransposons for plant breeding. Genome Biol 2017;18:134.
[91] Lanciano S, Carpentier MC, Llauro C, Jobet E, Robakowska-Hyzorek D, et al. Sequencing the extrachromosomal circular mobilome reveals retrotransposon activity in plants. PLoS Genet 2017;13(2):e1006630.
[92] Levan A, Levan G. Have double minutes functioning centromeres? Hereditas 1978;88(1):81–92.
[93] Kanda T, Wahl GM. The dynamics of acentric chromosomes in cancer cells revealed by GFP-based chromosome labeling strategies. J Cell Biochem Suppl 2000;35:107–14.
[94] Kanda T, Otter M, Wahl GM. Mitotic segregation of viral and cellular acentric extrachromosomal molecules by chromosome tethering. J Cell Sci 2001;114 (Pt 1):49–58.
[95] Simpson K, McGuigan A, Huxley C. Stable episomal maintenance of yeast artificial chromosomes in human cells. Mol Cell Biol 1996;16(9):5117–26.
[96] Marechal V, Dehee A, Chikhi-Brachet R, Piolot T, Coppey-Moisan M, et al. Mapping EBNA-1 domains involved in binding to metaphase chromosomes. J Virol 1999;73(5):4385–92.
[97] Baiker A, Maercker C, Piechaczek C, Schmidt SB, Bode J, et al. Mitotic stability of an episomal vector containing a human scaffold/matrix-attached region is provided by association with nuclear matrix. Nat Cell Biol 2000;2(3):182–4.
[98] Gaubatz JW. Extrachromosomal circular DNAs and genomic sequence plasticity in eukaryotic cells. Mutat Res 1990;237(5–6):271–92.
[99] Zhu Y, Gujar AD, Wong CH, Tjong H, Ngan CY, et al. Oncogenic extrachromosomal DNA functions as mobile enhancers to globally amplify chromosomal transcription. Cancer Cell 2021;39(5):694–707.
[100] Adelman K, Martin BJE. ecDNA party bus: Bringing the enhancer to you. Mol Cell 2021;81(9):1866–7.
[101] Helmsauer K, Valieva ME, Ali S, Chamorro González R, Schöpflin R, et al. Enhancer hijacking determines extrachromosomal circular MYCN amplicon architecture in neuroblastoma. Nat Commun 2020;11(1):5823.
[102] Morton AR, Dogan-Artun N, Faber ZJ, MacLeod G, Bartels CF, et al. Functional enhancers shape extrachromosomal oncogene amplifications. Cell 2019;179 (6):1330–41.
[103] Chapman OS, Luebeck J, Wani S, Tiwari A, Pagadala M, et al. The landscape of extrachromosomal circular DNA in medulloblastoma. bioRxiv 2021. https://doi.org/10.1101/2021.10.18.464907.
[104] Lange JT, Rose JC, Chen CY, Pichugin Y, Xie L, et al. The evolutionary dynamics of extrachromosomal DNA in human cancers. Nat Genet 2022;54:1527–33.
[105] Wang Y, Wang M, Djekidel MN, Chen H, Liu D, et al. eccDNAs are apoptotic products with high innate immunostimulatory activity. Nature 2021;599:308–14.
[106] Marichal T, Ohata K, Bedoret D, Mesnil C, Sabatel C, et al. DNA released from dying host cells mediates aluminum adjuvant activity. Nat Med 2011;17:996–1002.
[107] Li L, Olvera JM, Yoder KE, Mitchell RS, Butler SL, et al. Role of the non-homologous DNA end joining pathway in the early steps of retroviral infection. EMBO J 2001;20(12):3272–81.
[108] Chan CN, Trinité B, Lee CS, Mahajan S, Anand A, et al. HIV-1 latency and virus production from unintegrated genomes following direct infection of resting CD4 T cells. Retrovirology 2016;13:1.
[109] Engelman A, Englund G, Orenstein JM, Martin MA, Craigie R. Multiple effects of mutations in human immunodeficiency virus type 1 integrase on viral replication. J Virol 1995;69(5):2729–36.
[110] Ansari-Lari MA, Donehower LA, Gibbs RA. Analysis of human immunodeficiency virus type 1 integrase mutants. Virology 1995;211 (1):332–5.
[111] Wu Y, Marsh JW. Selective transcription and modulation of resting T cell activity by preintegrated HIV DNA. Science 2001;293(5534):1503–6.
[112] Gillim-Ross L, Cara A, Klotman ME. Nef expressed from human immunodeficiency virus type 1 extrachromosomal DNA downregulates CD4 on primary CD4+ T lymphocytes: implications for integrase inhibitors. J Gen Virol 2005;86(Pt 3):765–71.
[113] Swingler S, Mann A, Jacqué J, Brichacek B, Sasseville VG, et al. HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. Nat Med 1999;5(9):997–1103.
[114] Kaufman RJ, Brown PC, Schimke RT. Amplified dihydrofolate reductase genes in unstably methotrexate-resistant cells are associated with double minute chromosomes. PNAS 1979;76(11):5669–73.
[115] Haber DA, Beverley SM, Kiely ML, Schimke RT. Properties of an altered dihydrofolate reductase encoded by amplified genes in cultured mouse fibroblasts. J Biol Chem 1981;256(18):9501–10.
[116] Haber DA, Schimke RT. Unstable amplification of an altered dihydrofolate reductase gene associated with double-minute chromosomes. Cell 1981;26(3 Pt 1):355–62.
[117] Brown PC, Beverley SM, Schimke RT. Relationship of amplified dihydrofolate reductase genes to double minute chromosomes in unstably resistant mouse fibroblast cell lines. Mol Cell Biol 1981;1(12):1077–83.
[118] Kaufman RJ, Brown PC, Schimke RT. Loss and stabilization of amplified dihydrofolate reductase genes in mouse sarcoma S-180 cell lines. Mol Cell Biol 1981;1(12):1084–93.
[119] Nunberg JH, Kaufman RJ, Schimke RT, Urlaub G, Chasin LA. Amplified dihydrofolate reductase genes are localized to a homogeneously staining region of a single chromosome in a methotrexate-resistant Chinese hamster ovary cell line. PNAS 1978;75(11):5553–6.
[120] Dolnick BJ, Berenson RJ, Bertino JR, Kaufman RJ, Nunberg JH, et al. Correlation of dihydrofolate reductase elevation with gene amplification in a homogeneously staining chromosomal region in L5178Y cells. J Cell Biol 1979;83(2 Pt 1):394–402.
[121] Biedler JL, Melera PW, Spengler BA. Specifically altered metaphase chromosomes in antifolate-resistant Chinese hamster cells that overproduce dihydrofolate reductase. Cancer Genet Cytogenet 1980;2 (1):47–60.
[122] Raymond E, Faivre S, Weiss G, McGill J, Davidson K, et al. Effects of hydroxyurea on extrachromosomal DNA in patients with advanced ovarian carcinomas. Clin Cancer Res 2001;7(5):1171–80.
[123] Schoenlein PV, Barrett JT, Kulharya A, Dohn MR, Sanchez A, et al. Radiation therapy depletes extrachromosomally amplified drug resistance genes and oncogenes from tumor cells via micronuclear capture of episomes and double minute chromosomes. Int J Radiat Oncol Biol Phys 2003;55(4):1051–65.
[124] Rao VK, Wangsa D, Robey RW, Huff L, Honjo Y, et al. Characterization of ABCG2 gene amplification manifesting as extrachromosomal DNA in mitoxantrone-selected SF295 human glioblastoma cells. Cancer Genet Cytogenet 2005;160(2):126–33.
[125] Yu L, Zhao Y, Quan C, Ji W, Zhu J, et al. Gemcitabine eliminates double minute chromosomes from human ovarian cancer cells. PLoS ONE 2013;8(8):e71988.
[126] Kondo S, Barnett GH, Hara H, Morimura T, Takeuchi J. MDM2 protein confers the resistance of a human glioblastoma cell line to cisplatin-induced apoptosis. Oncogene 1995;10(10):2001–6.
[127] Bozic I, Allen B, Nowak MA. Dynamics of targeted cancer therapy. Trends Mol Med 2012;18(6):311–6.
[128] Sabnis AJ, Bivona TG. Principles of resistance to targeted cancer therapy: lessons from basic and translational cancer biology. Trends Mol Med 2019;25 (3):185–97.
[129] Sarmento-Ribeiro AB, Scorilas A, Gonçalves AC, Efferth T, Trougakos IP. The emergence of drug resistance to targeted cancer therapies: Clinical evidence. Drug Resist Updat 2019;47:100646.
[130] Nathanson DA, Gini B, Mottahedeh J, Visnyei K, Koga T, et al. Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA. Science 2014;343(6166):72–6.
[131] Song K, Minami JK, Huang A, Dehkordi SR, Lomeli SH, et al. Plasticity of extrachromosomal and intrachromosomal BRAF amplifications in overcoming targeted therapy dosage challenges. Cancer Discov 2022;12 (4):1046–69.
[132] Koo DH, Molin WT, Saski CA, Jiang J, Putta K, et al. Extrachromosomal circular DNA-based amplification and transmission of herbicide resistance in crop weed *Amaranthus palmeri*. PNAS 2018;115(13):3332–7.
[133] Hull RM, King M, Pizza G, Krueger F, Vergara X, et al. Transcription-induced formation of extrachromosomal DNA during yeast ageing. PLoS Biol 2019;17 (12):e3000471.
[134] Roers A, Hiller B, Hornung V. Recognition of endogenous nucleic acids by the innate immune system. Immunity 2016;44(4):739–54.
[135] Qiu GH, Zheng X, Fu M, Huang C, Yang X. The decreased exclusion of nuclear eccDNA: from molecular and subcellular levels to human aging and age-related diseases. Ageing Res Rev 2021;67:101306.
[136] Pan MR, Li K, Lin SY, Hung WC. Connecting the dots: from DNA damage and repair to aging. Int J Mol Sci 2016;17(5):685.
[137] Li Y, Zheng Q, Bao C, Li S, Guo W, et al. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. Cell Res 2015;25 (8):981–4.
[138] Lin H, Yu J, Gu X, Ge S, Fan X. Novel insights into exosomal circular RNAs: Redefining intercellular communication in cancer biology. Clin Transl Med 2021;11(12):e636.
[139] Wang G, Liu W, Zou Y, Wang G, Deng Y, et al. Three isoforms of exosomal circPTGR1 promote hepatocellular carcinoma metastasis via the miR449a-MET pathway. EBioMedicine 2019;40:432–45.

[140] Xu Y, Leng K, Yao Y, Kang P, Liao G, et al. A circular RNA, cholangiocarcinoma-associated circular RNA 1, contributes to cholangiocarcinoma progression, induces angiogenesis, and disrupts vascular endothelial barriers. Hepatology 2021;73(4):1419–35.
[141] Shimizu N, Shimura T, Tanaka T. Selective elimination of acentric double minutes from cancer cells through the extrusion of micronuclei. Mutat Res 2000;448(1):81–90.
[142] Balaj L, Lessard R, Dai L, Cho YJ, Pomeroy SL, et al. Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. Nat Commun 2011;2:180.
[143] Yokoi A, Villar-Prados A, Oliphint PA, Zhang J, Song X, et al. Mechanisms of nuclear content loading to exosomes. Sci Adv 2019;5(11):eaax8849. https://doi.org/10.1126/sciadv.aax8849.
[144] Moschoi R, Imbert V, Nebout M, Chiche J, Mary D, et al. Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy. Blood 2016;128(2):253–64.
[145] Marlein CR, Zaitseva L, Piddock RE, Robinson SD, Edwards DR, et al. NADPH oxidase-2 derived superoxide drives mitochondrial transfer from bone marrow stromal cells to leukemic blasts. Blood 2017;130(14):1649–60.
[146] Sansone P, Savini C, Kurelac I, Chang Q, Amato LB, et al. Packaging and transfer of mitochondrial DNA via exosomes regulate escape from dormancy in hormonal therapy-resistant breast cancer. PNAS 2017;114(43):E9066–75.
[147] Teo I, Veryard C, Barnes H, An SF, Jones M, et al. Circular forms of unintegrated human immunodeficiency virus type 1 DNA and high levels of viral protein expression: association with dementia and multinucleated giant cells in the brains of patients with AIDS. J Virol 1997;71(4):2928–33.
[148] Sharkey ME, Teo I, Greenough T, Sharova N, Luzuriaga K, et al. Persistence of episomal HIV-1 infection intermediates in patients on highly active anti-retroviral therapy. Nat Med 2000;6(1):76–81.
[149] Buzón MJ, Massanella M, Llibre JM, Esteve A, Dahl V, et al. HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. Nat Med 2010;16(4):460–5.
[150] Sharkey M, Babic DZ, Greenough T, Gulick R, Kuritzkes DR, et al. Episomal viral cDNAs identify a reservoir that fuels viral rebound after treatment interruption and that contributes to treatment failure. PLoS Pathog 2011;7(2):e1001303.
[151] Sharkey M, Triques K, Kuritzkes DR, Stevenson M. In vivo evidence for instability of episomal human immunodeficiency virus type 1 cDNA. J Virol 2005;79(8):5203–10.
[152] Cen Y, Fang Y, Ren Y, Hong S, Lu W, et al. Global characterization of extrachromosomal circular DNAs in advanced high grade serous ovarian cancer. Cell Death Dis 2022;13:342.
[153] Zhu J, Chen S, Zhang F, Wang L. Cell-free eccDNAs: a new type of nucleic acid component for liquid biopsy? Mol Diagnosis Therapy 2018;22(5):515–22.
[154] Wu X, Li P, Yimiti M, Ye Z, Fang X, et al. Identification and characterization of extrachromosomal circular DNA in plasma of lung adenocarcinoma patients. Int J Gen Med 2022;15:4781–91.
[155] Wei SH, Liu M, Hu J, Zhang CY. Target-initiated cascade signal amplification lights up a G-Quadruplex for a label-free detection of circular ribonucleic acids. Anal Chem 2022:9193–200. https://doi.org/10.1021/acs.analchem.2c01901.
[156] Umekage S, Uehara T, Fujita Y, Suzuki H, Kikuchi Y. In vivo circular RNA expression by the permuted intron-exon method. Innov Biotechnol 2012:75–90.
[157] Sin STK, Ji L, Deng J, Jiang P, Cheng SH, et al. Characteristics of fetal extrachromosomal circular DNA in maternal plasma: methylation status and clearance. Clin Chem 2021;67(5):788–96.
[158] Coenen-Stass AML, Pauwels MJ, Hanson B, Martin Perez C, Conceição M, et al. Extracellular microRNAs exhibit sequence-dependent stability and cellular release kinetics. RNA Biol 2019;16(5):696–706.
[159] Wang C, Liu H. Factors influencing degradation kinetics of mRNAs and half-lives of microRNAs, circRNAs, lncRNAs in blood in vitro using quantitative PCR. Sci Rep 2022;12:7259.
[160] Gao N, Li Y, Li J, Gao Z, Yang Z, et al. Long non-coding RNAs: the regulatory mechanisms, research strategies, and future directions in cancers. Front Oncol 2020;10:. https://doi.org/10.3389/fonc.2020.598817598817.
[161] Clark MB, Johnston RL, Inostroza-Ponta M, Fox AH, Fortini E, et al. Genome-wide analysis of long noncoding RNA stability. Genome Res 2012;22(5):885–98.
[162] Shi K, Liu T, Fu H, Li W, Zheng X. Genome-wide analysis of lncRNA stability in human. PLoS Comput Biol 2021;17(4):e1008918.
[163] Zhao X, Shi L, Ruan S, Bi W, Chen Y, et al. CircleBase: an integrated resource and analysis platform for human eccDNAs. Nucleic Acids Res 2022;50(D1):D72–82.
[164] Peng L, Zhou N, Zhang CY, Li GC, Yuan XQ. eccDNAdb: a database of extrachromosomal circular DNA profiles in human cancers. Oncogene 2022;41:2696–705.
[165] Yang M, Qiu B, He GY, Zhou JY, Yu HJ, et al. eccDB: a comprehensive repository for eccDNA-mediated chromatin contacts in multi-species. bioRxiv 2022. https://doi.org/10.1101/2022.09.22.509011.
[166] Foulkes I, Sharpless NE. Cancer grand challenges: embarking on a new era of discovery. Cancer Discov 2021;11(1):23–7.
[167] Fan Y, Mao R, Lv H, Xu J, Yan L, et al. Frequency of double minute chromosomes and combined cytogenetic abnormalities and their characteristics. J Appl Genet 2011;52(1):53–9.
[168] Mitelman F, Mertens F, Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer. 2016.
[169] Javadekar SM, Raghavan SC. Snaps and mends: DNA breaks and chromosomal translocations. Febs j 2015;282(14):2627–45.
[170] Burman B, Zhang ZZ, Pegoraro G, Lieb JD, Misteli T. Histone modifications predispose genome regions to breakage and translocation. Genes Dev 2015;29(13):1393–402.
[171] Kimmel M, Axelrod DE, Wahl GM. A branching process model of gene amplification following chromosome breakage. Mutat Res 1992;276(3):225–39.
[172] Pichugin Y, Huang W, Werner B. Stochastic dynamics of extra-chromosomal DNA. bioRxiv 2019. https://doi.org/10.1101/2019.12.15.876714.
[173] Lange JT, Chen CY, Pichugin Y, Xie L, Tang J, et al. Principles of ecDNA random inheritance drive rapid genome change and therapy resistance in human cancers. bioRxiv 2021. https://doi.org/10.1101/2021.06.11.447968.
[174] Barker PE, Drwinga HL, Hittelman WN, Maddox AM. Double minutes replicate once during S phase of the cell cycle. Exp Cell Res 1980;130(2):353–60.
[175] Shimizu N, Ochi T, Itonaga K. Replication timing of amplified genetic regions relates to intranuclear localization but not to genetic activity or G/R band. Exp Cell Res 2001;268(2):201–10.
[176] Balaban-Malenbaum G, Gilbert F. Double minute chromosomes and the homogeneously staining regions in chromosomes of a human neuroblastoma cell line. Science 1977;198(4318):739–41.
[177] Takayama S, Uwaike Y. Analysis of the replication mode of double minutes using the PCC technique combined with BrdUrd labeling. Chromosoma 1988;97(3):198–203.